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(54) **Improvements relating to the**
radio-labelling of proteins

(57) A method is provided for attaching
[32]P to a protein, normally an antibody,
that will bind with and irradiate a
tumour-associated structure leading to
therapeutic benefit. The invention is
based upon the introduction of a peptide
region to the protein, which is capable of
acting as a substrate for a
phosphokinase utilising [32]P—–ATP as a
typical source of the radionuclide. Novel
substrate peptides. Foxtide I and Foxtide
II are disclosed. Foxtide I is Lys. Tyr. Arg.
Arg. Ala. Ser. Leu. Gly. Foxtide II is Cys.
Arg. Arg. Lys. Ala. Ser. Gly. Pro. Pro. Val.

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TUMOUR LOCALISATION

FIG 1

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TUMOUR/PLASMA RATIO
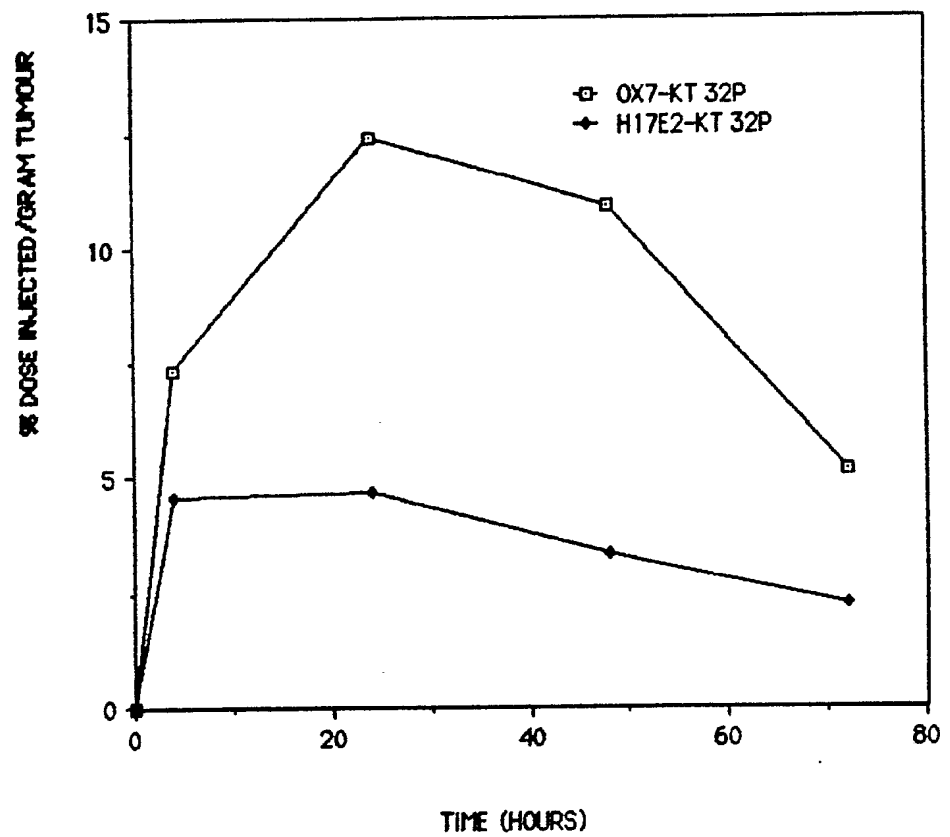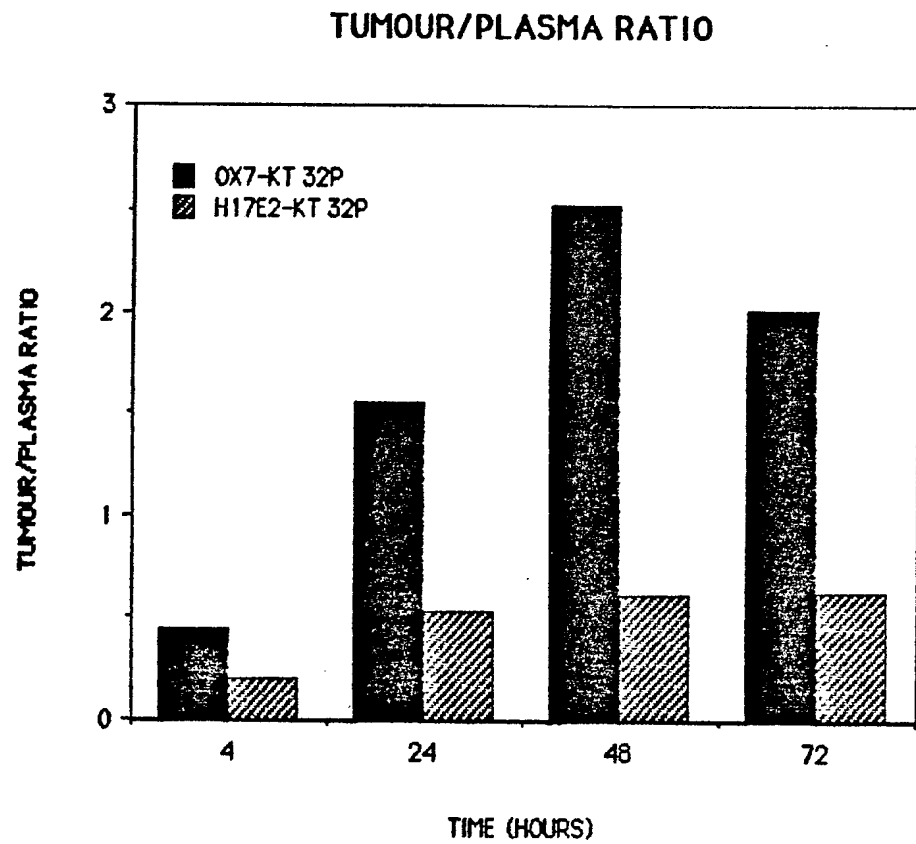

FIG 2

SPECIFICATION

Improvements relating to the radio-labelling of proteins

5   This invention relates to the radio-labelling of proteins and is specifically concerned with the labelling of monoclonal antibodies and other proteins with ^{32}P. The term "protein" as used herein
10   encompasses polypeptides.
        The concept of using monoclonal antibodies, and other proteins as delivery vehicles for the targeting of drugs is already established. Practical difficulties exist however when it comes to attaching the drug to
15   the monoclonal antibody or other delivery system since this must be done in such a way that the activity of the drug is retained and, at the same time, the specificity of the monoclonal antibody or other delivery system is maintained. At a practical level,
20   this places considerable restriction upon those chemical and biological methods theoretically available for the linking of the drug to the delivery system as many conventional reaction conditions will destroy either or both of the drug activity and
25   delivery system specificity.
        Radiation therapy is now well-established at one possible method of treatment of certain cancer conditions and the attachment of the radio-isotopes of iodine and a variety of metals, e.g. indium and
30   yttrium to antibodies is currently being investigated for this purpose. The radionuclide ^{32}P is, in many ways, a particularly advantageous radionuclide for use against certain types of solid tumours with relatively poor blood supply since ^{32}P has a
35   reasonably short half-life of 14 days and it is a pure beta-emitter with a particle energy of 1.7 MeV. However, it has not been possible to attach ^{32}P to antibodies by the methods that have been used previously for the attachment of other
40   radio-isotopes.
        We have now found a method by which a monoclonal antibody or similar targeting molecule can be structurally modified so that it can readily and swiftly have attached to it ^{32}P under mild reaction
45   conditions that maintain the specificity of the targeting molecule so as to give a ^{32}P labelled material.
        The present invention provides a method for modifying a protein that will bind with a
50   tumour-associated structure such as protein, glycolipid or carbohydrate, comprising the introduction into the binding protein of a peptide region, which is capable of acting as a substrate for a phosphokinase. The resulting modified binding
55   protein can then be ^{32}P labelled by reacting it with a ^{32}P-labelled gamma nucleotide triphosphate in the presence of a phosphokinase.
        This method gives rise to a binding protein (targeting molecule) carrying a ^{32}P label. Such ^{32}P
60   labelled compounds are new and form a further aspect of the present invention.
        The binding protein will normally be a monoclonal antibody that will bind with a tumour-associated antigen, for example antigens associated with solid
65   tumours with relatively poor blood supplies. Such

solid tumours include those found in the colon, ovaries and lungs and monoclonal antibodies to such tumour-associated antigens are already known and have already been used as delivery vehicles for
70   other anti-tumour agents. Such known antibodies can be linked to ^{32}P by the techniques of the present invention.
        More generally, the binding protein may be any protein that will bind with tumour-associated protein
75   (or other tumour-associated structure such as a glycolipid or carbohydrate) where the tumour is one susceptible to high-energy beta particles and, in addition to monoclonal antibodies, the first protein could be, for example, an Fab fragment or a hormone
80   or similar peptide that will bind to an appropriate receptor site identified on certain types of tumour cell e.g. melanocyte-stimulating hormone, epithelial growth factor, interferons and mitogenic peptides such as bombesin.
85   The present invention is based upon the structural modification of the binding protein to introduce a peptide region capable of acting as a substrate for the phosphokinase so that when the structurally modified "binding protein" which can be regarded
90   as "protein"/substrate conjugate is brought into contact with the ^{32}P containing phosphorylating agent in the presence of a phosphokinase, the enzyme can catalyse the transfer of ^{32}P from the phosphorylating agent into the substrate region of
95   the conjugate.
        Present availability points to the use of serine/threonine kinases as the phosphokinase. Such materials are now commercially available from the Sigma Company e.g. bovine heart protein kinase.
100   A hepta peptide known as Kemptide and having the structure Leu.Arg.Arg.Ala.Ser.Leu.Gly is now commercially available and is known to be a satisfactory substrate for the bovine heart protein kinase where phosphorylation with
105   gamma-^{32}P-adenosine triphosphate (ATP) results in ^{32}P phosphorylation at the serine residue of Kemptide. We have now surprisingly found that if the Kemptide structure is grafted onto a monoclonal antibody, not only is the specificity of the
110   monoclonal antibody unaffected but also that the ability of the monoclonal antibody/Kemptide conjugate to act as a substrate for the kinase is unimpaired and that phosphorylation from gamma-^{32}P-ATP can still proceed in a similar way to
115   phosphorylation of the heptapeptide molecule itself.
        One practical benefit of our new technique is that the monoclonal antibody or other protein can be partially prepared for phosphorylation by conjugation with the heptapeptide and the
120   phosphorylation left until immediately before the radio-labelled molecule is to be administered to the patient.
        The concept of the present invention does not depend upon the use of the specific heptapeptide
125   Kemptide and indeed, any peptide can be use provided it is capable of acting as a substrate for the phosphokinase. If the phosphokinase is the serine/threonine kinase derived from bovine heart as described in Example 4, then the main structural
130   requirement for the substrate molecule appears to

be that there be an area of positive charge, e.g.
arising from arginine and/or lysine residues, close to
the serine and/or threonine residues in the substrate.
Kemptide is one such substrate but we have worked
5 with other similar molecules in which we have
replaced the leucine residue at the N-terminus of
Kemptide by a lysine-tyrosine dipeptide to give an
octapeptide Lys.Tyr.Arg.Arg.Ala.Ser.Leu.Gly we
have called Foxtide I. The advantages of Foxtide I
10 over Kemptide are that conjugating the substrate
molecule to the antibody molecule is facilitated by
the existence of the lysine residue at the N-terminus
while the presence of the tyrosine molecule gives
ultra-violet "visibility" to the substrate molecule
15 facilitating purification and identification.
 We have also developed another substrate
molecule meeting the general requirements set out
above but which is decapeptide of the structure:
Cys.Arg.Arg.Lys.Ala.Ser.Gly.Pro.Pro.Val. We have
20 designated this decapeptide Foxtide II. Foxtide II has
advantages over Kemptide for our purposes in that
the serine residue can be phosphorylated more
quickly than can the serine residue in Kemptide
under otherwise similar reaction conditions using
25 gamma-^{32}P-ATP in the presence of bovine heart
protein kinase. Additionally, the cysteine residue at
the N-terminus facilitates conjugation with the
monoclonal antibody or other first protein through
the SH grouping in the terminal residue. Foxtide I
30 and Foxtide II may be prepared by conventional solid
state peptide synthesis on a Merrifield resin.
 Foxtide I and Foxtide II are new compounds and
these new compounds form part of the present
invention.
35 The substrate molecules discussed so far are all
substrates for serine/threonine kinases but other
types of phosphokinases are known and can be used
with the appropriate substrate. For example,
phosphokinases which are tyrosine kinases are
40 known and substrates for such tyrosine kinases are
known in which the enzyme brings about
phosphorylation of the tyrosine residue. Examples
of such tyrosine kinases include these contained in
Lymphoma cell extracts as described by Casnellie *et*
45 *al*, PNAS, 1982, *79*, 282-6. An example of a substrate
for the tyrosine kinases is:
 Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-Arg-Gln-Gly.
 The kinase substrates used in the present
invention can be of any molecular size. The tendency
50 is to use a substrate which is as small as possible
since the only requirement is to have a
phosphorylatable residue, e.g. serine, threonine or
tyrosine which, depending upon the enzyme being
used, may need to be in a close relationship to the
55 area of positive charge. Practicalities such as the cost
of synthesis and ease of purification will therefore
point to the use of small peptides containing up to
about 20 amino acid residues but larger substrates
could be used, bearing in mind that the larger the
60 substrate the more likely it is to interfere with the
properties of the final first protein/substrate
conjugate.
 Chemical methods can be used for the conjugation
of the substrate molecule to the monoclonal
65 antibody or other binding protein. Essentially, it is

necessary to bring about activation to an appropriate
level of reactive groupings in the first protein and in
the substrate molecule so that the necessary bonds
can be formed so as to bring about conjugation
70 while, at the same time, avoiding the use of reaction
conditions that will cause modification of the
specificity of the first protein in relation to the
tumour associated protein and the capacity of the
substrate molecule to act as a substrate during the
75 subsequent phosphokinase phosphorylation.
 We have found that satisfactory linking of the
substrate molecule to the targeting molecule (the
binding protein) can be achieved using appropriate
heterobifunctional protein crosslinking agents. For
80 example carboxylate] and SIAB
[N-succinimidyl-(4-iodoacetyl)aminobenzoate], all of
which are commercially available.
 The conjugates formed by linking the first protein
(i.e. the targeting molecule) to the substrate
85 molecule through a bridging group originating from
the heterobifunctional crosslinking agent are
themselves new compounds and form a further
aspect of the present invention. In addition to the
conventional synthetic methods described above for
90 producing the protein/substrate conjugate, the
appropriate substrate sequence may be integrated
into an appropriate position within the primary
structure of the first protein by using genetic
engineering techniques and the use of such
95 techniques is also within the scope of this invention.
 The targeting molecule/substrate molecule
conjugates are stable materials that can be stored for
prolonged periods of time at room temperature or
slightly below, e.g. 0 to 20°C. We have found that,
100 after conjugation of the targeting molecule with the
substrate molecule using hetero-bifunctional protein
crosslinking agents, the specificity of the targeting
molecule and the substrate capacity of the substrate
molecule is retained both initially and after
105 prolonged storage at 0 to 20°C.
 One of the benefits of the present invention is that
the targeting molecule/substrate molecule
conjugate can be stored in this form for prolonged
periods of time prior to use and that it can be easily
110 phosphorylated to introduce the ^{32}P function
immediately prior to use in a patient. The
phosphorylation itself can be carried out by any
conventional phosphokinase/phosphorylating agent
system and we have found that satisfactory results
115 can be readily achieved e.g. in periods of about 30
minutes, using ^{32}P gamma-ATP (e.g. the material
sold as product Code PB10218 by Amersham
International Limited, England) with bovine heart
protein kinase and a phosphorylatable serine or
120 threonine residue in the substrate portion of the
conjugate. As an alternative to ^{32}P-gamma-ATP, one
can also use ^{32}P-gamma-guanidine triphosphate as
phosphorylating agent used in association with the
appropriate phosphokinase. These phosphorylating
125 agents may also be used in association with the
appropriate phosphokinases to introduce ^{32}P onto
the tyrosine residue in conjugates having a tyrosine
residue in the substrate portion.
 Although the present invention is primarily
130 designed to facilitate enzymatic phosphorylation of

the phosphorylatable amino acid residues in the substrate portion of the conjugate, chemical phosphorylation would also be possible.

Once the phosphorylation of the targeting
5 molecule/substrate molecule conjugate has been completed, the ^{32}P labelled material can be purified from inorganic materials by standard chromatographic techniques such as gel filtration, e.g. on a Sephadex column equilibrated with
10 phosphate buffered saline. The ^{32}P conjugate solution obtained in this way may then be filtered (0.22 u) and is then in a suitable form for administration.

According to a further feature of the invention, we
15 provide a pharmaceutical composition, particularly one for parenteral administration, comprising a pharmaceutically acceptable diluent, and a ^{32}P-labelled protein that will bind with a tumour-associated structure.
20 Once a trace dose of radiolabelled binding protein is shown to target preferentially for a tumour as compared to normal tissue, then the ^{32}P-labelled binding protein may be given to the subject intravenously or into various body regions i.e. by
25 intraperitoneal, intrapleural or intra-arterial infusion.

The use of the ^{32}P-labelled binding protein according to the invention has the advantage that the procedures for protection during handling and preparing the radioactive products are simplified,
30 external radiation doses to staff are reduced and storage and disposal problems are simplified by the non-volatile nature of the material. ^{32}P has advantages over other radioactive isotopes, for example ^{131}I, in that a patient can receive a higher
35 dose rate with ^{32}P-labelled binding protein since the relative activity concentration required to deliver a specific dose to a target is less than that required for ^{131}I; the unit dose delivered to a particular target tissue using phosphorous labelled targeting
40 molecules is, for example, approximately twice that delivered when using ^{131}I labelled targeting molecules. Moreover the effects of this radiation therapy on uninvolved tissues are reduced significantly by the absence of penetrating gamma
45 radiation.

Damage to uninvolved tissues may be minimised by administration of ^{31}P-orthophosphate salts for a period of several weeks following treatment with the ^{32}P-labelled binding protein.
50 The most sensitive normal tissue is the bone marrow and the application of marrow transplantation to allow the use of very high doses of ^{32}P-labelled binding protein.

The ^{32}P-labelled binding proteins are of interest in
55 the treatment of, for example, ovarian cancer, colon metastases to the liver, malignant pleural effusions and brain tumours.

The following Examples are given to illustrate the way in which the invention can be put into practice
60 using, as targeting molecules, monoclonal antibodies that will bind to solid tumours implanted in mice.

EXAMPLE 1
65 Introduction of an iodoacetyl group into the

peptide receptor molecule "Kemptide" (Leu.Arg.Arg.Ala.Ser.Leu.Gly).

N-Succinimidyl-2-iodoacetate (0.75 mg, 2 eq.) in dry dimethyl formamide (DMF, 62.5 ul) was added to
70 a solution of "Kemptide" (1.5 mg) in water (60 μl) which had first been diluted with methanol (40 μl). After incubation for one hour at room temperature, the reaction was shown to be complete by analysis of a sample with thin layer chromatography (TLC) (6065
75 Cellulose plates, Eastman, eluting with 1-butanol: water: acetic acid: pyridine in the proportions 50: 40: 2: 32 v/v) - ninhydrin staining demonstrated the removal of the free primary amino groups. The reaction mixture was then used directly for coupling
80 to the thiopropylated antibody as described in Example 2.

EXAMPLE 2
Introduction of a thio group into OX7 antibody and
85 subsequent coupling with iodacetyl "Kemptide".
A solution of N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) (44 μl of a stock solution of 3.1 mg/ml in dry DMF) was added to a solution of OX7 monoclonal antibody
90 (7.8 mg) in borate buffer (1.0 ml, 0.05 M sodium borate containing 0.1M sodium chloride and 0.5% v/v 1-butanol; pH 9.0). The molar ratio of SPDP to immunoglobulin was 8:1. After incubation at room temperature for one hour, the reaction mixture was
95 desalted on a G50 'Sephadex' column (60 ml) which had been equilibrated in acetate buffer (0.1 M sodium acetate containing 0.1 M sodium chloride and 1 mM ethylene diamine tetraacetic acid (EDTA); pH 4.5). Analysis of the eluted protein by the
100 standard method of Carlsson et al., (Biochem J. 1978, 173, 723) revealed that an average of 4.6 dithiopropyl groups had been introduced per IgG molecule. The protein solution (5.6 ml) was then incubated with dithiothreitol (275 μl of molar stock solution to give a
105 final concentration of 50 mM) for one hour at room temperature and then desalted on a G50 'Sephadex' column (60 ml) equilibrated in nitrogen-flushed phosphate buffer (0.1 M disodium hydrogen phosphate buffer (pH 7.5) containing 0.1 M sodium
110 chloride and 1 mM EDTA). The eluted protein was immediately concentrated again by 'Amicon' ultrafiltration to 1.0 ml, 6.3 mg/ml), diluted with DMF (200 μl) and treated with the iodacetylated Kemptide solution (30 μl, prepared as described in Example 1).
115 This gave a final ratio of 2.5 iodacetyl residues per thiopropyl group. The reaction mixture was incubated at room temperature for 24 hours and any remaining unreacted thiol groups were then blocked by the addition of a solution of N-ethylmaleimide (5
120 mg) in DMF (100 £1). After a further hour, the reaction mixture was applied to a G50 'Sephadex' column (60 ml) equilibrated in the "enzyme buffer" (50 mM potassium hydrogen phosphate (pH 7.0) containing 5 mM magnesium chloride and 0.25 mM EGTA
125 [ethyleneglycol-bis-(beta-aminoethyl ether)-N,N,N^1,N^1-tetraacetic acid]) and the eluted protein concentrated to 1 mg/ml by 'Amicon' ultrafiltration, filtered (0.22 μ) and stored at 4°C. The number of 'Kemptide' groups conjugated to each
130 antibody molecule by this procedure was shown to

be about 2.0 by trace-labelling a sample of the product with ^{32}P.

EXAMPLE 3

5 Retention of antibody function by OX7-'Kemptide' conjugate
Solutions of OX7-Kemptide conjugate (50 ul, prepared as described in Example 2) at various concentrations were added to aliquots of AKR-A
10 mouse lymphoma cells (1 ml at 10^6 cells/ml) in phosphate buffered saline (PBS) containing bovine serum albumen (BSA) (2 mg/ml) and sodium azide (0.05%). After incubation at 37°C for 30 minutes, the cells were washed twice with the PBS solution and
15 the resultant cell pellets treated with fluorescein isothiocyanate-labelled rabbit anti-mouse antibody (Miles Labs.), diluted 1:32 from stock. After incubation for 30 minutes at 37°C, the cells were washed in PBS/BSA/azide solution and finally
20 suspended in 1 ml of the buffer solution. Flow cytometry analysis of at least 10^4 cells at each concentration showed that conjugate and native OX7 had identical binding characteristics and there was no evidence of a decrease in affinity of the
25 conjugated OX7. 50% saturation of the binding sites was achieved at about 60 ng/ml of OX7 or OX7-'Kemptide'.

EXAMPLE 4

30 *Phosphorylation of OX7-'Kemptide'*
For high specific activity labelling, OX7-'Kemptide' stock solution (70 µl at 1 mg/ml, prepared as described in Example 2) and x 5 "enzyme buffer" (30 µl, 250 mM dipotassium hydrogen phosphate (pH
35 7.0) containing 25 mM magnesium chloride and 1.25 mM EGTA) was added to 1 mCi of 32P-γ-ATP (adenosine triphosphate) (100 µl, PB10218, Amersham International), followed by bovine heart protein kinase (5 µl, 50 U, Sigma). The reaction was
40 incubated for 30 minutes at 37°C and the protein was then desalted using a G50 'Sephadex' column (10 ml) equilibrated in phosphate-buffered saline which had been pre-washed in phosphate-buffered saline containing bovine serum albumin (2 mg/ml). Under
45 these conditions, about 0.15 phosphate moieties were incorporated into each molecule of OX7 with a specific activity of 5.26 µCi/ug.

EXAMPLE 5

50 *Preparation of a conjugate from iodoacetyl 'Kemptide' and H17E2*
H17E2 is a monoclonal antibody raised against alpha-placental alkaline phosphatase which is normally found in placenta but is also expressed by
55 ovarian, testicular, cervical and glioma tumour tissue.
This conjugate was prepared in essentially the same way as Example 2 but using H17E2 (10 mg), SPDP (170 µg) dithiothreitol (65 µl of molar solution)
60 and iodoacetyl-'Kemptide' (67 µl of the solution, prepared as described in Example 1). Seven thiol groups were introduced to the antibody by this procedure and a three-fold excess of iodoacetyl-Kemptide was used to maximise
65 coupling. Any remaining unreacted thiol was then

blocked with N-ethylmaleimide (6 mg) in DMF (120 µl). Labelling with trace amounts of ^{32}P showed that there was an average of about 4.52 sites on each antibody molecule that could be labelled with ^{32}P.
70 Labelling at high-specific activity as described in Example 4 gave a product with a specific activity of 5.23 µCi/ug.

EXAMPLE 6

75 *Preparation of a conjugate from iodoacetyl 'Foxtide' and the monoclonal antibody OX7*
N-Succinimidyl-2-iodoacetate (0.28 mg, 1 eq) in dry DMF (14 µl) was added to a solution of 'Foxtide I' (Lys.Tyr.Arg.Arg.Ala.Ser.Leu.Gly) (1 mg) in water (40
80 µl) diluted with methanol (60 µl) which was then treated with 100 µM sodium hydroxide (14 µl) giving a pH of 6.4. After incubation for one hour at room temperature, TLC followed by ninhydrin staining indicated that the reaction was complete. A sample
85 of this reaction mixture (60 µl) was added to a solution of thiolated OX7 monoclonal antibody (650 µl, prepared as described in Example 2) and incubated at 4°C for 72 hours. Unreacted thiol groups (if any) were then blocked by the addition of
90 N-ethylmaleimide (5 mg) in DMF (50 µl) and after incubation for one hour, the conjugate was isolated and stored in the enzyme buffer as described in Example 2. The number of Foxtide I groups conjugated to each antibody molecule by this
95 procedure was shown to be 1.32 by trace-labelling a sample of the product with ^{32}P.

EXAMPLE 7
Stability of phosphorylated conjugates of OX7 in
100 *human, mouse and rat plasma.*
1M disodium hydrogen phosphate buffer solution (125 µl, pH 7.19) and penicillin/streptomycin (6 µl, Flow Labs Cat. No. 16-700-49) was added to 500 µl samples of fresh plasma from human, mouse (nu nu)
105 and rat (Sprague-Dawley) sources. The samples were then sterile-filtered (0.22 µ) and each treated with an equal volume of a sterile solution of Ig-Kemptide-^{32}P (450 µl at 225 µg/ml and 200 mCi/mmole Ig prepared as described in Example 4
110 but at a lower specific activity). The mixtures were incubated at 37°C and triplicate samples (20 µl) were taken at a range of time points over a 64 hour period. Acid precipitates were obtained, collected on glass fibre filters and counted but showed no loss of
115 radioactivity during the course of the experiment.

EXAMPLE 8
Comparison of the affinities of the conjugate H17E2-Kemptide-^{32}P for LOVO cells and T47-D cells.
120 Solutions of H17E2-Kemptide-^{32}P conjugate (50 µl, prepared as descrined in Example 5) at a range of concentrations were added to aliquots of LOVO (a human colorectal cancer cell line which expresses alpha-placental alkaline phosphatase) or T47-D cells
125 (a human breast cancer cell line that does not express the alkaline phosphatase) at 10 cells/450 µl medium and incubated at 37°C for one hour. After washing the cells, the amounts of radioactivity retained were measured. With the LOVO cells, the
130 binding ranged from 2.60% at 10 ng/500 µl to 0.36%

at 500 ng/500 μl while there was no measurable
binding to the T47-D cells (0.05%). The viability of the
LOVO cells was reduced by 40-55% with 1-100 ng of
conjugate/assay and by 75% at 500 ng/assay.

5   EXAMPLE 9
Synthesis of Foxtide I
    Foxtide I (the octapeptide Lys. Tyr. Arg. Arg. Ala.
Ser. Leu. Gly) was synthesised using the "Classical"
10 SPPS Merrifield chemistry on an "Applied
Biosystems 430A Peptide Synthesiser".

EXAMPLE 10
Synthesis of Foxtide II
15   Foxtide II (the decapeptide Cys. Arg. Arg. Lys. Ala.
Ser. Gly. Pro. Pro. Val.) was synthesised using the
"Classical" SPPS Merrifield chemistry on an
"Applied Biosystems 430A Peptide Synthesiser".

20 EXAMPLE 11
*Localisation of OX7-Kemptide-^{32}P to subcutaneously
implanted AKR A Lymphoma*
    Solutions of either OX7-Kemptide-^{32}P conjugate
(0.2 ml, 6.9 μCi) or H17E2-Kemptide-^{32}P conjugate
25 (0.2 ml, 8.0 μCi) were injected intravenously into nu
nu mice which had been previously implanted
subcutaneously with AKR A lymphoma cells (3 × 10^6
per mouse). The AKR A lymphoma has surface
receptors which bind OX7 antibody. The mice were
30 killed and blood samples taken following the
removal of the tumours. Measurement of the
radioactivity in the tissues (Figure 1) showed that the
maximum accumulation of the OX7-conjugate by the
tumour occurred within 24 hours. This was followed
35 by a steady loss of label from the tumour which
dropped by half over the following 48 hour period.
However, the OX7 conjugate clears from the
bloodstream faster as the increasing plasma ratios
indicate (Figure 2). The apparent tumour uptake of
40 the non-specific H17E2 conjugate is largely due to
the blood circulating through the tumour.

CLAIMS

45   1. A method for modifying a protein that will bind
with a tumour-associated structure such as protein,
glycolipid or carbohydrate, comprising the
introduction into the binding protein of a peptide
region, which is capable of acting as a substrate for a
50 phosphokinase.
    2. A method according to claim 1 wherein the
peptide substrate is attached to the binding protein
by chemical means.
    3. A method according to claim 1 wherein the
55 peptide substrate is incorporated in the binding
protein by genetic engineering techniques.
    4. A method according to any one of the
preceding claims in which the peptide substrate is
Kemptide (Leu. Arg. Arg. Ala. Ser. Leu. Gly).
60   5. A method according to any one of claims 1 to 3
in which the peptide substrate is Foxtide I (Lys. Tyr.
Arg. Arg. Ala. Ser. Leu. Gly).
    6. A method according to any one of claims 1 to 3
in which the peptide substrate is Foxtide II (Cys. Arg.
65 Arg. Lys. Ala. Ser. Gly. Pro. Pro. Val).

    7. A method according to any one of the
preceding claims in which the modified binding
protein is reacted with a ^{32}P-γ-labelled nucleotide
triphosphate in the presence of a phosphokinase to
70 give a ^{32}P-labelled modified binding protein.
    8. A method according to claim 7 in which the
triphosphate is ^{32}P-γ-adenosine triphosphate.
    9. A method according to any one of the
preceding claims wherein the binding protein is an
75 antibody that binds with a tumour-associated
antigen.
    10. A method according to claim 1 substantially
as hereinbefore described in any one of the
Examples.
80   11. Foxtide I (Lys. Tyr. Arg. Arg. Ala. Ser. Leu.
Gly).
    12. Foxtide II (Cys. Arg. Arg. Lys. Ala. Ser. Gly,
Pro. Pro. Val.).
    13. A ^{32}P-labelled protein capable of binding with
85 a tumour associated antigen.
    14. A pharmaceutical composition comprising a
pharmaceutical acceptable carrier and a ^{32}P-labelled
protein according to claim 13 or obtained by a
method according to any one of claims 7 to 10.
90   15. A composition according to claim 14 for
parenteral administration.
    16. A composition according to claim 14 or 15 for
use in a method of therapy or in a method of
diagnosis.